1194-Plat
The Nature of Membrane Curvature-Induction by Amphipathic α-Helices Relies upon Protein Length: Simulations of α-Synuclein and H0
Anthony R. Braun[1], Eva Sevcsik[2], Elizabeth Rhoades[2], Stephanie Tristram-Nagle[3], Jonathan N. Sachs[1].
[1]University of Minnesota, Minneapolis, MN, USA, [2]Yale University, New Haven, CT, USA, [3]Carnegie Mellon University, Pittsburgh, PA, USA.
Remodeling of membranes, for example the induction of membrane curvature, is a necessary step in vesicle fusion and fission. One mechanism for curvature induction relies upon the interfacial binding of amphipathic proteins, which act as a wedge in one leaflet of the membrane. In this study, we explore differences between the membrane remodeling effects of two biologically important amphipathic proteins, specifically α-Synuclein (αS) and the N-BAR helix H0. αS is an intrinsically disordered protein that adopts an amphipathic α-helical structure upon binding a membrane. Recently it has been shown that αS can induce tubulation and vesiculation of vesicles (Varkey et al. 2010, J Biol Chem, 285(42):32486). In contrast, the amphipathic helix H0 is unable, on its own, to induce tubulation, as N-BAR relies instead upon its large scaffolding domain (Fernandes et al. 2008, Biophys J, 94(8):3065). Using a combination of x-ray scattering and coarse-grained molecular dynamics simulations, we explored the membrane remodeling effects of αS. Our findings suggest that αS 1) causes a significant thinning of the bilayer; and 2) stabilizes an anisotropic curvature-field of both positive mean curvature and negative Gaussian curvature. Simulations of H0 show a similar magnitude of the local curvature as with αS, however owing to its molecular length the curvature-field around H0 is isotropic. We propose that the difference in curvature-field anisotropy explains the difference in the two proteins' abilities to induce macroscopic curvature (i.e. tubulation) of lipid vesicles.

1195-Plat
Computer Simulation of Membrane Tubulation by EFC F-BAR Domain Lattices
Hang Yu, Klaus Schulten, Ying Yin, Anton Arkhipov.
University of Illinois, Urbana Champaign, Urbana, IL, USA.
Cells are dynamically sculpted into many types of compartments by cellular membranes, in some cases with the help of BAR domain proteins. BAR domain proteins act under in vitro conditions are found to induce formation of tubules. We have seen in coarse-grained molecular dynamics simulation stretching over 100 microseconds how a flat membrane is curved into a tube when F-BAR domain proteins are arranged on the membrane surface as a regular lattice of parallel rows. The simulations could also characterize the membrane bending properties of F-BAR domains in different lattice arrangements, showing membrane curvatures with radii ranging from 25 to 100 nm.
Lastly, the simulations reveal two key structural features of F-BAR domain that facilitate efficient binding to membranes and membrane curving: (1) Curving is promoted by close contact between phosphoserine lipid head groups and clusters of cationic residues along the membrane facing surface of F-BAR domains, namely lysine and arginine residues 30, 33, 110, 113, 114, and 139, 140, 146, 150, respectively. (2) Within the 100 ns of contact, the F-BAR domain hinge region, through a 20 degree rotation of the helix moment of inertia, establishes a close contact between protein and membrane. (1) and (2) result in membrane bending on a microsecond-to-millisecond time scale.

1196-Plat
Invisible Binding: DnaA, the Initiator of Chromosome Replication in Bacteria, Associates with the Escherichia Coli Inner Membrane In Vivo
Tomer Regev, Nadav Myers, Raz Zarivach, Itzhak Fishov.
Ben Gurion University of the Negev, Beer-Sheva, Israel.
DnaA initiates chromosome replication in most known bacteria and its activity is controlled to execute only once every cell division cycle. ATP in the active ATP-DnaA is hydrolyzed after initiation and ADP is replaced back to ATP on the verge of next initiation. Thus DnaA acts as a molecular switch, in which the nucleotide recycling couples key processes in the cell. Two putative recycling mechanisms presume binding of DnaA either to the membrane or to specific chromosomal sites, promoting nucleotide dissociation. While there is no doubt that DnaA interacts with artificial membranes *in vitro*, it is still controversial as to whether it binds the cytoplasmic membrane *in vivo*. We sought after DnaA-membrane interaction in *E. coli* cells employing fluorescent microscopy and cell fractionation with both native and fluorescent DnaA hybrids. A small (5-10%) but reliable portion of DnaA is indeed membrane associated, though invisible in fluorescent cell images. This small fraction is physiologically significant as representing the free DnaA available for initiation. Using combination of mCherry with variety of DnaA fragments, we demonstrate that the membrane binding function is delocalized on the protein structure. Analysis of *E. coli* DnaA structure model reveals a hydrophobic continuity on the protein surface, supporting a concerted interaction of distant residues with the membrane, rather than by an individual amphiphilic helix. A binding-bending mechanism is suggested, explaining the membrane-induced nucleotide release from DnaA. We have suggested previously that the enigmatic 'initiation mass' phenomenon may result from a highly cooperative interconversion between two functional states of DnaA driven by its membrane surface occupancy (Aranovich et al., 2006, Aranovich et al., 2007). Our present results provide a strong basis for extrapolation of this phenomenon to *in vivo* situation.

1197-Plat
Curvature as a Mechanism for Biomolecule Localization in Bacterial Cells
Lars D. Renner[1], Prahathees Eswaramoorthy[2], Kumaran S. Ramamurthi[2], Douglas B. Weibel[1].
[1]University of Wisconsin Madison, Madison, WI, USA, [2]National Cancer Institute, Bethesda, MD, USA.
One of the central questions in cell biology is how the temporal and spatial organization of the cell machinery within the cell is established, maintained, and replicated. In Eubacteria, an understanding of the cellular organization of proteins is just beginning to take shape. Numerous, functionally unrelated proteins have been found that localize to regions of rod-shaped bacterial cells that are characterized by a high intrinsic curvature (e.g. the poles and the division septum). Recent data suggests that there are geometric cues for the localization of proteins and lipids in bacteria. We have recently tested the hypothesis that membrane anisotropy occurs by mechanisms governed by physical and geometrical constrains. We found that microdomains of cardiolipin (CL) preferentially localize to regions of large, negative curvature. In this presentation we explore whether these domains or curvature are responsible for protein localization. We present data for the localization of two functionally bacterial division proteins, MinD (from *Escherichia coli*) and DivIVA (from *Bacillus subtilis*) that localize to regions of large curvature in vivo. We use a top-down approach that combines in vivo and in vitro experiments with *E. coli* and *B. subtilis* cells. We find that a critical difference in the radius of curvature ΔC (curvature difference between cell poles and midcell) of ~0.5 μm^{-1} is required to drive the polar localization of MinD and DivIVA. Our data provides support for curvature as a general mechanism for regulating the spatial organization in bacterial membranes. This research expands our understanding of Eubacterial cell biology and provides insight into the spatial and temporal dynamics of membranes and their role in cell biology.

Platform: Actin & Actin-binding Proteins

1198-Plat
Coarse-Grained Analysis and Modeling of Nucleotide-Dependent Changes in F-Actin
Marissa G. Saunders, Gregory A. Voth.
Department of Chemistry, Institute for Biophysical Dynamics, James Franck Institute, and Computation Institute, University of Chicago, Chicago, IL, USA.
Actin filaments represent a truly multiscale physical system in which changes at the chemical reaction level (ATP hydrolysis) affect the conformation of actin subunits which in turn affect the binding affinity of actin-binding proteins and the stability of actin filaments. Connecting information between these scales and understanding the physical basis of these changes is a significant and challenging problem.
We present a coarse-grained (CG) model and analysis of molecular dynamics simulations of actin filaments in the ATP and ADP-bound states to understand the large scale changes in structure and dynamics based on nucleotide state. Because the CG model is based on the underlying protein structure, changes in the distribution and stability of CG sites highlight specific areas in the filament that may respond to the nucleotide state, and which should be analyzed further at the atomistic scale. These CG models are essential to allow simulation of actin filaments at a network level and to characterize how nucleotide state affects the emergent properties of the network.